CONTRIBUTIONS TO OUR KNOWLEDGE OF SOIL-FERTILITY.

No. xiii. The Toxicity of Soils.

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In previous communications, I have shown that when a soil is shaken up with water in the proportion of 100 grams of soil to 100 c.c. of water, there is obtained an extract which, under certain conditions, is toxic towards bacteria. The bacteria may be very sensitive to the toxin, such for example as *Bac. prodigiosus*, or they may be slightly sensitive, such as a mixture of the soil-bacteria. The toxic effect is shown in the case of *Bac. prodigiosus* as a distinct diminution in the number of bacteria, and, in the soil-bacteria, as a retardation of the speed of the increase.

In the soil-extracts, there are present nutritive as well as toxic substances, and the direct evidence of the action of the toxin in destroying bacteria will naturally depend upon the presence of a preponderating amount. Otherwise, if the nutritive substances are in excess, no direct evidence of toxic action will be obtained. It is easy to demonstrate an indirect toxic action. One has only to prepare a soil-extract, to heat one portion, and to compare the rate of growth of bacteria added to each part. It will generally be found that all heated soil-extracts, when seeded with bacteria, will produce a greater number in, say, twenty-four hours than an unheated but otherwise similar extract. The reason for this is that the toxins have been destroyed, and, with these out of the way, the growth of the bacteria is much increased. The destruction of the toxins by heat may be analogous to the conversion of the petroleum-soluble and preservative hop-constitu-

ents into petroleum-insoluble and non-preservative substances upon being boiled with water.*

The presence of the toxin in the soil depends upon the natural conditions to which the soil has been subjected. Rain, for example, washes it out, and the extract, instead of being toxic, is nutritive. The decay of the toxin in the soil is accelerated by dry conditions, so that in dry, as in wet weather, we cannot expect to find the extracts toxic. There is a medium between wet and dry conditions which, while probably not influencing the actual formation of the toxin, yet facilitates our being able to demonstrate its presence.

There is still much to be learned regarding the nature of the toxic solution and of the production of toxins. Several matters relating to these have been investigated, and are here recorded.

The relation of the quantity of Water used in extracting the Soil —It is curious that the quantity of water used for extraction should have an influence in enabling the toxic effect to be evident, and although this has been shown to occur,† yet the difficulty of explaining the phenomenon caused another test to be made, when time should have modified any personal factor.

A garden-soil, seven days after heavy rain, gave the following result : –

	Cells of *Bac. prodigiosus* in 20 hours at 22°.
Bacteria in control-test	100
Bacteria in extract	40

Two days later, when the soil contained 7·05% of moisture, it was shaken up with varying amounts of water and extracts prepared. These were seeded with a suspension of *Bac. prodigiosus*,

* Chapman, Proc. Chem. Soc., xxix. (417), p.182.
† These Proceedings, 1914, pp.729-731.

and incubated at 22°. Next day, plates were prepared and the bacteria counted.

100 grams of soil to	Cells of *Bac. prodigiosus* in 20 hours at 22°.
30 c c. of water	94
50 c c. of water	176
75 c.c. of water	175
100 c.c. of water	67
400 c c. of water	60
Control-test, water only	100

The results are in keeping with previous tests, in which it was shown that the general tendency was for the extract to be toxic when comparatively small quantities of water relatively to the soil were used; with larger quantities, the extracts became nutritive, but again became toxic at about the 1:1 ratio.

It is difficult to use less than 30 c.c. of water per 100 grams of soil in preparing an extract. In order, therefore, to obtain information regarding the possibility of toxic action in soil-water, the test-bacteria were added to soil directly with varying quantities of water. The test-bacteria are thus in competition with the soil-bacteria, and the conditions are not the same as when extracts are used. At the same time, it has been shown that the presence of soil- and test-bacteria in extracts do not affect the general trend of the toxic or nutritive effect, as indicated by the decrease or increase of the test-bacteria. So far as the test-bacteria are concerned, the soil-bacteria are relatively inert, during the time of the experiment.

Twenty-gram portions of soil were put into petri-dishes, and 1 c.c. of a suspension of *Bac. prodigiosus* was added to each along with varying quantities of water. The soils were thoroughly mixed with the water, and the dishes were put into a damp-chamber and incubated at 28° for 20 hours. They were then transferred to flasks of sterile water, shaken, diluted, and $\frac{1}{40}$ c.c. smeared on agar-plates, dried at 37°, incubated, and counted.

For further information regarding the soils, an extract was made, and the numbers E/C were obtained by dividing the in-

crease during 20 hours at 22° in the extract by the numbers in the control-test. A soil which gives an extract that is neither nutritive nor toxic, shows a growth ratio, E/C = 1, a nutritive soil is greater than 1, and a toxic soil is less.

The numbers in the tables are compared with 100 bacteria added at the start or originally present For the actual numbers, the following are taken from experiments *c* and *d*.

	Bacteria at start in millions per gram of dry soil.	
	Bac. prodigiosus.	Soil-bacteria.
Expt. *c*	0·233	6·074
Expt. *d* .	0·545	7·551

100 bacteria in 20 hours at 20° became

a		*b*		*c*			*d*		
Moisture.	*Bac. prodigiosus.*	Moisture.	*Bac. prodigiosus.*	Moisture.	*Bac. prodigiosus.*	Soil-bacteria.	Moisture.	*Bac. prodigiosus.*	Soil-bacteria.
8·3	120	9·3	70	7·8	—	232	7·3	—	73
12·5	98	13·5	52	12·2	102	166	11·7	72	99
16·2	99	17·2	51	16·1	151	155	15·7	79	119
19·7	95	20·6	47	19 8	69	143	19·4	65	121
23·0	100	23·8	40	23·1	81	141	22·7	45	99
26·0	96	26·8	38	26·2	56	132	25·8	43	107
28·6	99	29·5	37	29·1	53	109	28·7	43	80
—	—	—	—	31·7	53	114	31·3	36	90
E/C = 1.		E/C = 0·04.		E/C = 0·75.			E/C = 60.		

There are two points in the experiments that are not quite clear. These are, (1) the jump of the soil-bacteria in the $\frac{3}{4}$ toxic soil (Expt. *c*) from 100 to 232 upon incubating at 28° for 20 hours, and the fall of the soil-bacteria in the nutritive soil (Expt. *d*); (2) the diminution of *Bac. prodigiosus* in a soil which gives a nutritive extract (Expt. *d*).

The general tendency is for the bacteria in the soils to decrease with an increase in the moisture-content. This may have been caused by the water gradually displacing the air in the soil-spaces, but, as the non-toxic and non-nutritive soil of Expt. *a* did not show this tendency, we may be justified in assuming that there was a sufficiency of air under the conditions of the experiments, viz., a shallow layer of soil of about 2 mm. thickness. It appears that an increase in the amount of moisture increases the toxic effect of the soil-water.

When the results of these experiments are considered in conjunction with those of the experiments with soil-extracts, it is seen that the effect of adding water in progressive amounts to the soil is to cause a certain degree of toxicity to become manifest. As the quantity of water relative to the soil is increased, the toxicity becomes less marked. This is when the ratio of soil to water is about 1 : 0·5. Then the toxicity increases, and is generally most pronounced when the ratio is 1 : 1. In one case,* this occurred at the 1 : 1·5 ratio. With the addition of more water, the toxicity diminishes.

Moisture and Temperature in Formation of Toxin.—In attempting to produce bacteriotoxins in soils in the laboratory, one realises that certain conditions should favour their production. The moisture-content of the soil and the temperature are clearly of outstanding importance. An attempt was accordingly made to investigate these.

A soil was divided into 200 gram portions, and water was added to bring the moisture-contents up to 5, 10, 15, and 20%. Three sets were prepared and stored in bottles in the laboratory. Stumps of matches were set at the sides of the corks to ensure a limited communication with the outside air. At intervals, each set was taken, and the soils shaken up with 200 c.c. of 0·2% of potassium sulphate (added to hasten filtration), and due allowance was made for the water present as moisture. The extracts were, as usual, filtered through paper and porcelain, and seeded

* These Proceedings, 1913, p.773, Experiment xi.

with a suspension of *Bac. prodigiosus*, incubated for 20 hours at 22°, and counted by the plate-method.

	Number of cells of *Bac. prodigiosus*.		
	17 days.	32 days.	41 days.
Water-control	100	100	100
Extract of soil with 5% moisture	252	45	115
Extract of soil with 10% moisture	82	96	68
Extract of soil with 15% moisture	142	102	75
Extract of soil with 20% moisture	218	145	86

The water-control was the same dilute potassium sulphate which was used for making the extracts, and, like the extracts, it had been filtered through porcelain to ensure sterility. This is necessary because sterilisation by heat alters the toxic or nutritive effect of the water.

While the extract from the 5% soil was variable, those from the 15% and 20% showed a steady increase of toxicity. The 10% extract was always toxic.

Having determined that about 10% of moisture, or one quarter of the H.W.C., was best for obtaining a soil furnishing a toxic extract, an attempt was made to determine the optimum temperature. For this purpose, the soil was taken soon after rain, when it should have been either feebly toxic or nutritive. Two hundred gram portions were put into bottles, which were corked and incubated at four different temperatures for varying times. At the start, the soil contained 11·05% of moisture.

	Number of cells sf *Bac. prodigiosus* in 20 hours at 22°.			
	2 days.	9 days.	14 days.	26 days.
Water-control	100	100	100	100
Extract of soil, 15°	114	130	60	166
Extract of soil, 22°	107	90	59	169
Extract of soil, 28°	106	90	37	20
Extract of soil, 37°	557	130	40	43

Considering the results generally, it is seen that 15° to 22° give very similar results, and that 28° is the optimum tempera-

ture for a rapid development of toxin. The highest temperature, 37°, appears to show that an initial destruction of toxin occurred, and that this was followed by a subsequent formation.

Extracts from chloroformed and air-dried soils.—As a rule, the extract of a soil which has been treated with a volatile antiseptic, such as chloroform, is more nutritive towards bacteria than untreated soil. The amount of moisture in the soil when chloroformed should have some influence, and the nature of the result may depend upon whether the soil has been chloroformed in the natural state, or whether it has been more or less air-dried to obtain a more representative sample. That the moisture has an influence was shown in an experiment in which the soil, with its natural moisture, was passed through a sieve with 13 meshes to the inch, and then treated with 5% of chloroform before and after being air-dried. The disinfectant was aired-off after two days' contact, and extracts of the soils were made in the manner which has been previously described. The extracts were seeded with a suspension of *Bac. prodigiosus*, and incubated for 21 hours at 22° and counted.

	Bacterial growth.
Water-control	100
Extract of soil, chloroformed wet	33,000
Extract of soil, chloroformed dry	216,080

The experiment shows that the moisture in the soil hinders the action of the chloroform in liberating nutritive materials from the soil.

The effect of air-drying alone upon the toxic or nutritive nature of the extract obtained from the soils was investigated upon three occasions.

	Growth of *Bac. prodigiosus*.		
	Sept. 1st.	Sept. 5th.	Sept. 9th, 1914.
Water-control	100	100	100
Extract of moist soil... ..	16	103	87
Extract of air-dried soil ...	3	3,730	5,500

The results indicate that if the soil is strongly toxic, as on September 1st, air-drying increases the toxicity of the extract;

but if it is feebly toxic, as on September 9th, or virtually non-toxic, as on September 5th, drying brings about a greater solubility of the nutrients if it does not destroy the bacteriotoxins.*

The number of soil-bacteria in the soil was tested on September 5th, and found to be as follows :—

Raw soil ... 14 millions per gram of dry soil.
Air-dried soil... 3·6 millions per gram of dry soil.

The Development of Soil-Toxins.—Although the development of soil bacteriotoxins has been previously described in these Proceedings, some further work was done during the later months of 1914 by way of confirmation. The garden-soil under grass was passed through a coarse sieve (No.13), and moistened with a small quantity of water to bring the content up to 6%. It was then heated in the oven until a thermometer, with its bulb in the centre of the soil, registered 80° for 10 minutes. After cooling down, the moisture was made up to 10%, and the soil was incubated at 28°. As the soil was contained in an enamelled bucket with a lid, the moisture did not fall rapidly, and any loss was made good from time to time. Extracts were made in the ordinary manner, and these were seeded with *Bac. prodigiosus* and with a suspension of bacteria from the same bucket of soil.

	Bacteria in 1 gram of dry soil, millions.	Bacterial growth in 20 hours at 22°.	
		Bac. prodigiosus.	Soil-bacteria.
At start	1·0	—	—
6 days	77	1140	490
13 days	112	—	390
21 days	51	280	100
27 days	51	200	130
34 days	56	410	230
Water-controls ...	—	100	100

* Buddin (Journ. Agric. Sci., vi., 452) shows that the air-drying of soil causes an increase in the amount of nitrate formed during a period of three months under laboratory conditions; and that a drying to 98 % of dry matter gives a greater nitrate-increase than a drying to 96 %. In another experiment, in which the drying diminished the bacteria from 10 to 4 or 5 millions, no difference was detected in the amount of ammonia and nitrate immediately after treatment, but after an incubation of 4 months, the soils which had not been air-dried contained 28 to 33 parts of nitrate per million, and those which were air-dried contained 41 parts.

The growth of the bacteria in the extract was compared with the growth in the water used in preparing the extracts, both extract and water being primarily freed from bacteria by filtration through porcelain.

At the end of the experiment, the soil was tested for protozoa, and representatives of the three classes, ciliates, monads, and amœbæ, were found.

The experiment is similar to others in which the development of toxicity is not so marked as the lessened nutritive action. It is possible that the preliminary heating played some part in preventing the development of toxins in sufficient quantity to show a direct toxic action, such as was obtained in the moisture-experiment (p.636, *antea*).

Soils seem to develop toxicity much more readily under natural conditions than when bottled up in the laboratory. In casting about for a reason for a difference in the behaviour, one noted that the garden-soil had a covering of grass, which was absent in the experimental soil. This may be a reason for the difference, for the Duke of Bedford and Pickering have recently shown that the leachings of grassed soils are toxic to fruit-trees, to various plants, and to grass itself. They consider that these toxins are not secreted by the plant-roots directly, but result from the decay of the débris of the growing roots. Thus the bacteria are brought into the matter, and an increase of root-débris should mean a greater bacterial fermentation and increased amount of bacterial products, toxic to the bacteria themselves and to plants. Whether these two are the same or not, has yet to be determined, but, at present, these authors have indicated a relation between plant-growth and bacteriotoxins. This relation was to be expected, as any substance which increases bacterial growth will necessarily increase the bacterial by-products.

In commenting upon these experiments of the Duke of Bedford and Pickering, Miller* says, "It does not seem very clear why the leachings from the trays are injurious to the plants in the pots . . . . whilst it is without action, as soil-solution, before it drains out of its own pot." The leachings are really unfiltered

* Annual Reports Progress of Chemistry, Chem. Soc., 1914, 232.

soil-extracts, and my experiments have shown that the toxicity is only apparent when the ratio of water is about 1:1. It would be necessary to use this ratio in order to obtain leachings, for a smaller quantity of water, say a ratio of 1 : 0·5, would simply wet the soil. My experiments with bacteriotoxins have shown that the half ratio gives an extract which is nutritive to bacteria, just as it is to plants. The toxicity of the leachings and the beneficial effect of the simple wetting appear to bring the bacteriotoxins into line with the plant-toxins, and make it possible that they are similar, if not identical.

If the root-débris consists of the shrivelled root-hairs, it is a substance which will decay with comparative slowness, and we should expect very little toxin to be developed in a month, a usual time for laboratory experiments. This is really what occurs; a comparatively small amount of toxin does develop. But I have found that, in the open soil under grass, the production of toxin is much quicker, and this leads to the supposition that some quickly decomposing substance is given to the soil by the grass. Some years ago, Mazé* showed that a gramineous plant, maize, secreted dextrose. In water-culture experiments under aseptic conditions, he found this sugar in the water bathing the roots of his plants. If this is the case, for it has not been confirmed, we have a reason for the rapid production of toxin in the soil in which plants such as grass are growing.

It has been known for some time that crops are injuriously affected by the presence of easily fermentable carbohydrates in the soil. The deleterious action of raw farmyard-manure upon certain soils is an example. Russell showed that, unless time were given for starch to decay, its addition to soil was followed by a diminution of the crop. Lipman† showed that the addition of glucose to soil depressed the yield of crop even when an excess of fertilising material, including nitrate, was present, and, furthermore, that the depression of the crop was not due to the action of denitrification of the nitrate by bacteria or moulds, as nitrates were found in the affected crop.

* Annal. de l'Inst. Pasteur, xxv. (1911), p.724.

† Lipman, New Jersey Agric. Expt. Stn. Rept. No.257.

These considerations lead one to expect that the addition of glucose to soil will bring about a more rapid production of bacteriotoxin. The quantity must not be too great or there will not be a complete fermentation, and any left undecomposed in the soil will, upon extraction with water, act as a nutrient masking the toxic effect.

A preliminary experiment was made with an alluvial soil which had been in store for some time. It was subsequently considered that a raw soil would have been more suitable as probably containing a more diverse flora, but, unfavourable as the conditions were, the experiment showed that dextrose undoubtedly assisted the formation of bacteriotoxins. It was somewhat difficult to decide upon the amount of dextrose to add to the soil. Lipman obtained his toxic effects with 10, 20, and 30 grams of dextrose to 20 pounds of soil, a quantity roughly equivalent to 0·1%, 0·2%, and 0·3%. He got a pronounced diminution of crop with 0·2%. Mazé obtained 57 milligrams of glucose from a maize-plant growing in three litres of water. This is approximately equal to 0·02%. These quantities were given and obtained during a growing period, but, to obtain evidence of toxicity in a short time, a smaller amount would probably be better to ensure a complete fermentation.

The experimental soil was accordingly treated with 0·005% of dextrose, and check-tests without sugar were made at the same time. Two of the conditions for toxin-formation had already been determined. These are, that the soil should contain an amount of moisture equal to about one-quarter of the water-holding capacity, and that the temperature of incubation should be from 22° to 28°. As the laboratory temperature was about 25°, the bottles of soil were allowed to stand upon a laboratory-bench.

Bac. prodigiosus in 20 hours at 22°. Control-test = 10.

	4 days.	9 days.
No sugar	56	12
Dextrose 0·005%	23	1

The tests with no sugar behaved just like previous tests. The nutritive effect was reduced upon incubation, and, although this

could only occur through the production of bacteriotoxin, the toxic effect was not sufficient to show a direct toxicity. With dextrose, the case was different. The toxicity was so great as to completely overshadow any nutritive action on the ninth day. Further tests were made, but the results were irregular.

EXPERIMENT 12/11/14.

	Bac. prodigiosus; average control-test = 10.		
	4 days.	11 days.	18 days.
No sugar	18	98	8
Dextrose, 0·01% ..	84	100	12
Dextrose, 0·1% ...	172	21	5

EXPERIMENT 25/11/14.

	Bac. prodigiosus; average control-test = 10.		
	1 day.	8 days	15 days.
No sugar	16	28	8
Dextrose, 0·005% ...	19	31	3
Dextrose, 0·01% ...	15	43	6
Dextrose, 0·02% ...	17	23	8

It appeared possible that aëration of the soil might hasten the production of toxin, and, accordingly, a current of air was passed over the soil contained in bottles.

EXPERIMENT 18/12/14.

	Bac. prodigiosus; average control-test = 10. 4 days.	
——	Air passing.	No air passing.
No sugar	16	13
Dextrose, 0·005%	23	1

The aëration of the soil containing dextrose did not appear to assist the formation of toxin, and, accordingly, further tests

were made to confirm the result. These showed that the time of aëration was of importance.

	Bac. prodigiosus; average control-test = 10.		
		Air passing.	No air passing.
Dextrose, 0·005% ...			
8/2/15	1 day.	7	21
		6	26
25/2/15		21	11
		7	11
10/3/15		14	10
		20	13
25/2/15		15	23
		12	1
	average	13	18
Dextrose, 0·005% ...			
25/1/15	3 days	12	1
		14	2
15/2/15		19	15
		17	13
	average ..	15	8
Dextrose, 0·005% ...			
1/2/15	5 days	47	5
		15	4
Dextrose, 0·01%... ...			
16/3/15	2 days	9	16
		12	13
8/4/15	4 days	5	17
		23	17
23/3/15	6 days	1	13
		5	21

The duplicates of the tests are so irregular that conclusions regarding the effect of aëration cannot be definitely drawn.

But after making allowance for apparent discrepancies, it appears that, with the smallest amount of dextrose, viz., 0·005%, the passing of air over the soil makes it at first more toxic, then more nutritive. This is presumably caused by the decomposition or decay of toxin rapidly formed from the small quantity of sugar. With 0·01% of dextrose, aëration makes the soil toxic in about five days, with an air-temperature running about 25°. Simple storage of the soil containing the smaller amount of dextrose, results in the formation of toxin in five days, while with the larger quantity, a toxic effect does not become apparent in that time. On the whole, aëration appears to hasten the formation and destruction of toxins formed from the sugar.

These experiments with dextrose and soil show that the presence of small quantities of sugar undoubtedly increases the toxicity of the soil, when a sufficient time is given for the sugar to become decomposed. The determination of the time will naturally depend upon the conditions which govern microbic fermentation, and include the quantity of sugar, the aëration, the temperature, etc.

The preceding experiments were made with the test-organism, *Bac. prodigiosus*, which can give direct evidence of toxicity in soil-extracts. The soil-bacteria have not been found to do this; they show the toxicity indirectly by a diminished growth under the conditions of the experiments, as has been shown in previous papers. A diminished development of bacteria is also seen in the following experiment in which a rather large quantity of dextrose, viz., 0·1%, was added to the soil. This contained 8·3% of moisture, and was stored at laboratory-temperature. The bacteria used for infecting the extracts and water-controls were obtained by shaking some of the same soil with sterile water, and using the suspension. As in other experiments, the growth was determined by counting the bacteria after an incubation at 22° for 20 hours. (See Table on next page).

A diminution of the bacterial growth is evident, but, unfortunately, the gradual loss of nutritive effect, in the absence of a direct evidence of toxic action, may be capable of two explanations. The gradually diminished growth may be caused by the

production of toxin, or by the gradual loss, by decomposition, of the nutritive sugar added to the soil.

	Bacteria in millions per gram of dry soil.	Growth of soil-bacteria in the soil-extract; water-control = 10.
At start	17	302
8 days	84	122
28 days	163	109
57 days	47	84

It is concluded from the experimental work detailed in this paper that:—(1) The formation of toxins in the soil, free from vegetation, occurs most rapidly when the temperature is near 28°, and the moisture-content is one-fourth of the water-holding capacity.

(2) The soil-extract is, as a rule, either nutritive or toxic according to the volume of water, relative to the soil, used in preparing the extract. It is most nutritive when the ratio of soil to water is 1 : 0·5, and most toxic when it is 1 : 1.

(3) A previous drying or chloroforming of the soil generally causes the extract to be much more nutritive than when the raw soil is used.

(4) The addition of small quantities of dextrose to soil brings about a more rapid production of toxin, while aëration of the treated soil accelerates the formation and decay of the toxin.